"Consistency and hard work are key to success"

CSIR NET – Life Science

Unit 1: Biochemistry

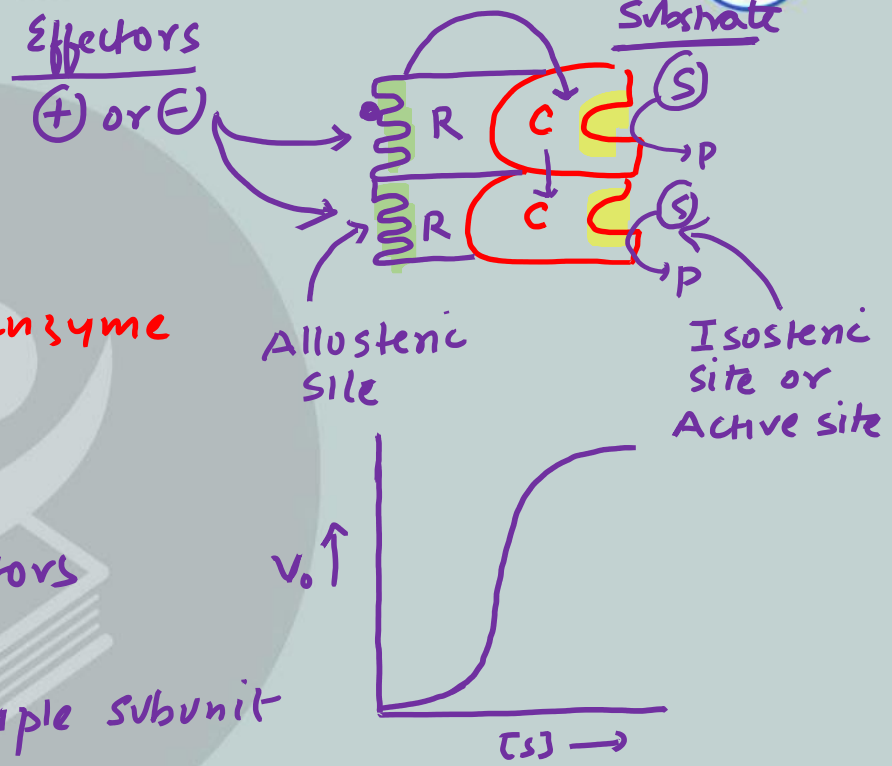
13 Enzyme Regulation and Metabolism

Points to be covered in this Lecture

- Allosteric Enzyme ✓
- Isoenzyme ✓
- Ribozyme ✓
- Regulation of Enzyme Activity ✓
- Enzyme Purification ✓
- Basics of metabolism ✓
- Glycolysis ✓

Allosteric Enzymes

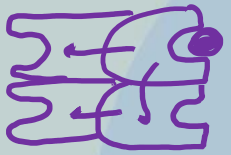
- Intracellular ✓
- Pacemaker ← Slow enzyme
- Catalytic + Regulatory subunit ✓
- Intrinsic regulation ← Effectors
- Cooperativity → multiple subunit
- Hill's equation → Donot obey mm equation

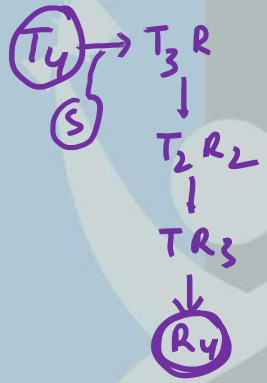


$V_{max} = K_{cat}\, E_T$

Hill's Equation and Plot

Describe the cooperative binding of substate to allosteric enzyme





$$V_0 = V_{max} \frac{[S]^n}{K_{0.5} + [S]^n}$$

n = Hill's coefficient

n value is more than 1

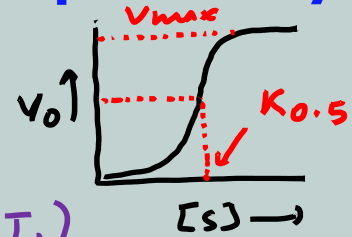
$K_m = K_{0.5}$

[S] required to achieve $V_0 = \frac{1}{2} V_{max}$

Cooperativity

Tense state (T_0)

Relax state (R_0)

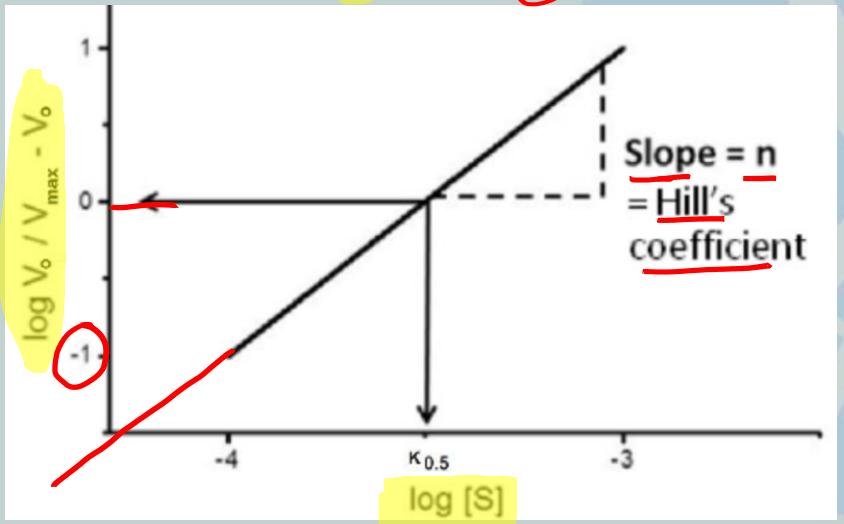


Straight Line

$V_0 = V_{max} \frac{[S]^n}{K_{0.5} + [S]^n}$

$$\frac{\log V_0}{V_{max} - V_0} = n \log [S] - \log K_{0.5}$$

$Y = m \cdot x - c$



X axis = log [S]

Y-axis = $\frac{\log V_0}{V_{max} - V_0}$

x-axis intercept = $\log K_{0.5}$

Slope = n (Hill's coefficient)

Allosteric constant (L)

The equilibrium constant for the transition between two forms of an allosteric protein in the absence of regulator

$$L = T_0 / R_0$$

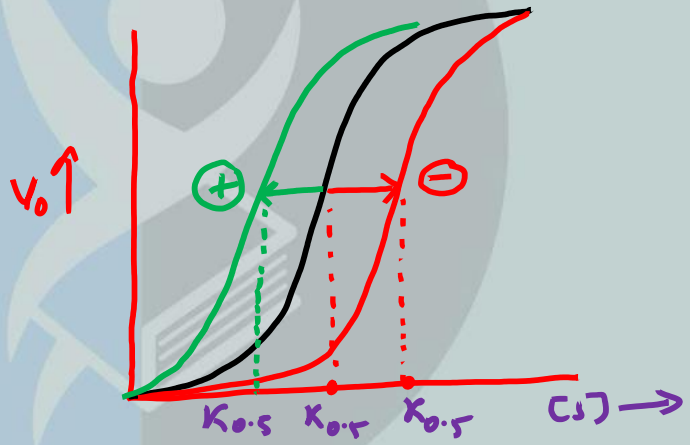
$$L = \frac{\text{Tense state}}{\text{Relax state}}$$

Tense state ← Inhibitor (−)

Relax state ← Activators (+)

Positive regulator: Decreases allosteric constant

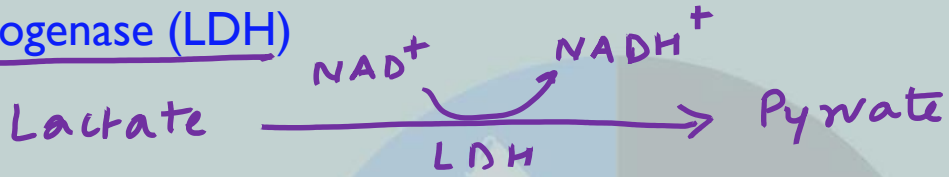
Negative regulator: Increases allosteric constant



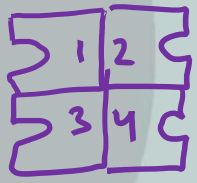
1. Isozymes or isoenzymes:

- Variants of the enzyme that exist co-functionally within the same organism
- Arise from different genes or from alternative splicing of the same gene.
- Differ in terms of molecular weight, optimal pH, kinetic parameters like Km and Kcat and regulatory mechanisms.
- Often expressed in specific tissues or at certain developmental stages.
- Allow for more complex regulation of metabolic pathways
- Can be markers for tissue damage or disease states

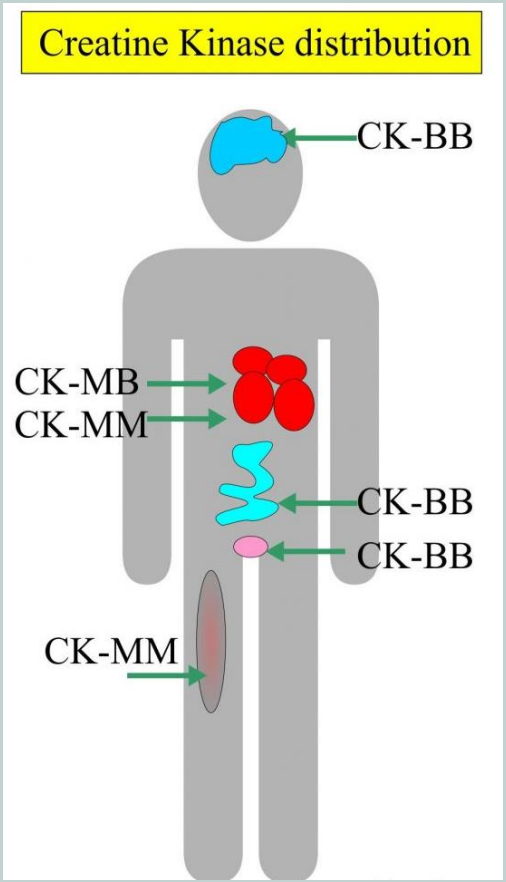
Lactate dehydrogenase (LDH)



- LDH is composed of four subunits, which can be of two types: H (heart) and M (muscle).
- The different combinations of these subunits form five isoenzymes:



- LDH-1 (H4): heart and red blood cells,
- LDH-2 (H3M1): reticuloendothelial system,
- LDH-3 (H2M2): in lungs,
- LDH-4 (H1M3): in kidneys, pancreas, and placenta,
- LDH-5 (M4): liver and skeletal muscle.



Creatine Kinase

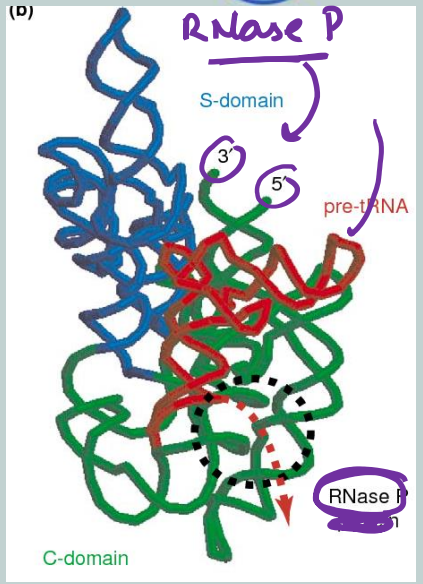
Energy Rich
↓
Creatine (P)

Creatine ⟷ Creatine (P)
ATP
ADP

- Dimer
- Two types of subunit M (Muscle) and B (Brain)
- CK has three isoenzymes
  - CK-MM: Found predominantly in skeletal muscle and heart.
  - CK-MB: Found in heart muscle and is of particular importance in diagnosing myocardial infarction.
  - CK-BB: Primarily present in the brain and smooth muscle, including the gastrointestinal tract.

Ribo-enzyme → Ribonucleic acid (RNA) act as enzyme

- Thomas Cech and Sidney Altman
- Catalytic RNA
- Ancestral enzymes — Limited conformations
- Slow and Unstable, Limited catalytic strategy (2'–OH)



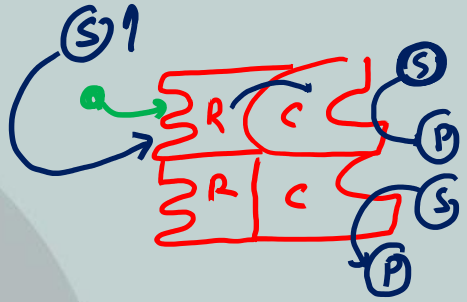
- **Hammerhead ribozyme**: Catalyzes its own cleavage during replication of viroids and virusoids.
- **Hairpin ribozyme**: Also involved in self-cleavage linked to replication processes.

Group I = Pre rRNA
Group II : Pre mRNA

- **Group I and Group II introns**: Catalyze their own excision from RNA transcripts and the ligation of the remaining exons.

- **RNase P**: Involved in the maturation of pre-tRNA by cleaving its 5' leader sequence.
- **Peptidyl transferase of the ribosome**: forms peptide bonds
  → 23 S rRNA (Prokaryotes)

REGULATION OF ENZYME ACTIVITY



1. Allosteric regulation : Allosteric enzyme

- **Homotropic effectors:** When substrate itself act as effector

  Hetrotropic effectors: Except substrate some other molecule act as effector

Binds to Allosteric site

Non-covalent interaction (Reversible)

S_1 S_2 Products

$F6P + ATP \xrightarrow{PFK-1} F16BP + ADP$

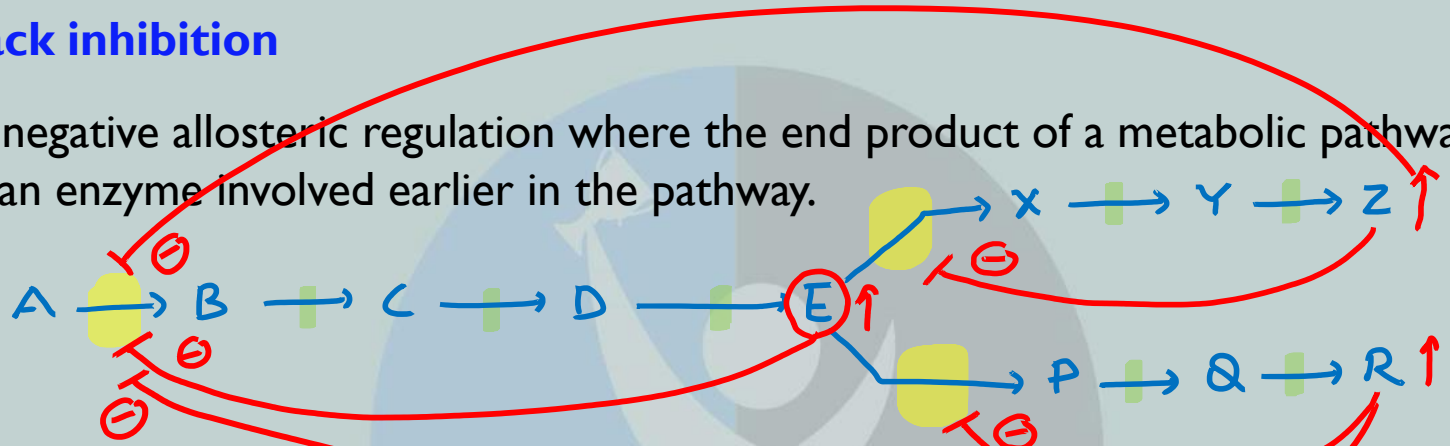
PFK-1: (+) F6P, ATP; (−) Inhibitor; (+) F26BP; (−)

F6P ATP — Homotropic

F26BP Inhibitor — Heterotropic

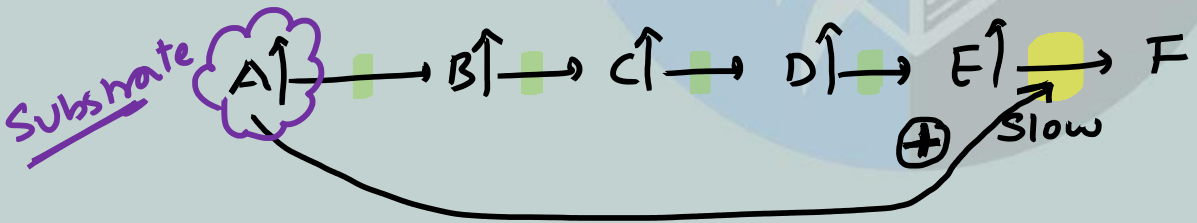
Feedback inhibition

Type of negative allosteric regulation where the end product of a metabolic pathway inhibits an enzyme involved earlier in the pathway.



Feed forward activation

Type of positive allosteric regulation where a metabolite produced early in a pathway enhances the activity of an enzyme later in the same pathway.

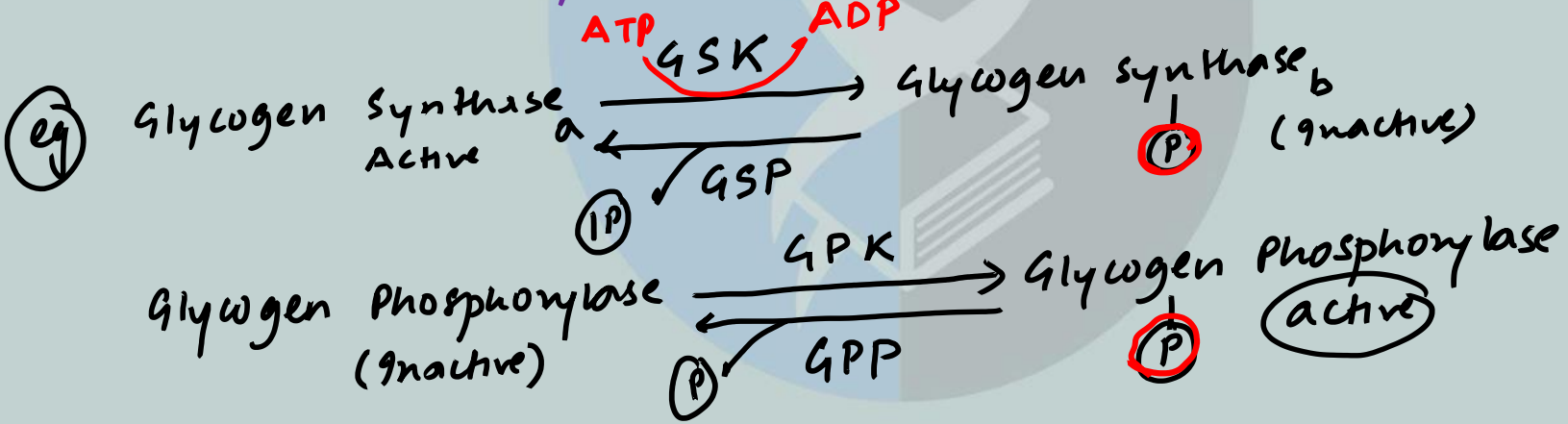


2. Regulation of enzymes by covalent modification

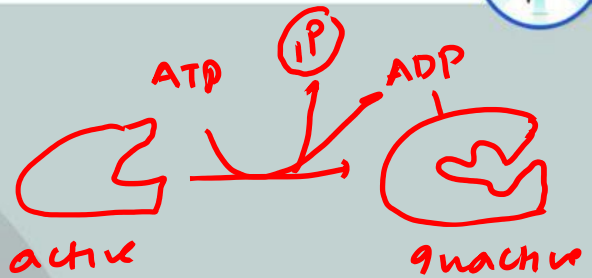
Reversible addition or removal of chemical groups to specific amino acids in an enzyme, thereby altering its activity, stability, or cellular location

- **Phosphorylation/Dephosphorylation:** → Ser, Tyr, His, Asp, Thr

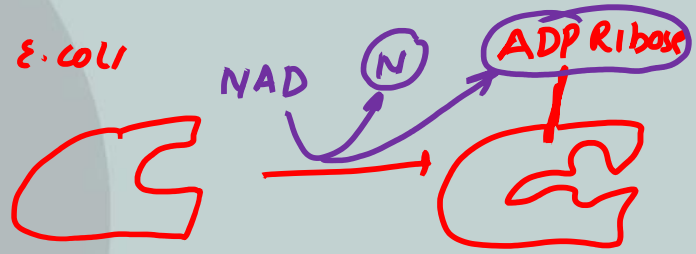
Kinase Phosphatase ← Regulatory enzyme.



- **Nucleotidylation of a specific Tyr** : Glutamine synthetase (*E . coli*).
  - Addition of nucleotide – ADP
  - Enzyme is inactivated

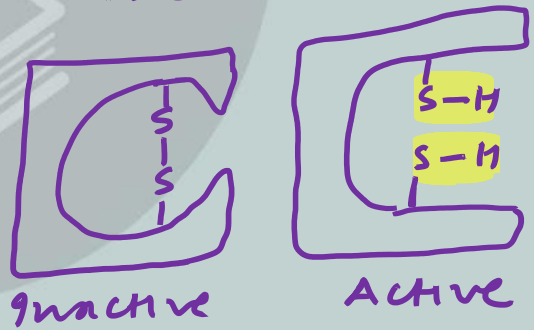


- **ADP-ribosylation of a specific Arg** : Adenylate cyclase of E. coli
  - Addition of ADP ribose from NAD
  - enzyme is inactivated



N = Nicotinamide

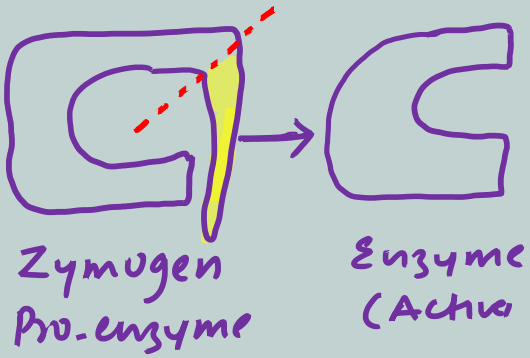
- **Disulphide reduction**
  - Reducing env^{r}
  - Disulphide bond break
  - Sulphahydral bond are formed
  - enzyme is activated



3. Proteolytic activation

- Irreversible
- Enzyme has short half life

- The precise cleavage of one or more specific peptide bonds within a zymogen.
- This cleavage induces a conformational change in the molecule, either by removing a segment that inhibits activity or by exposing a previously hidden active site.
- Cascading Events

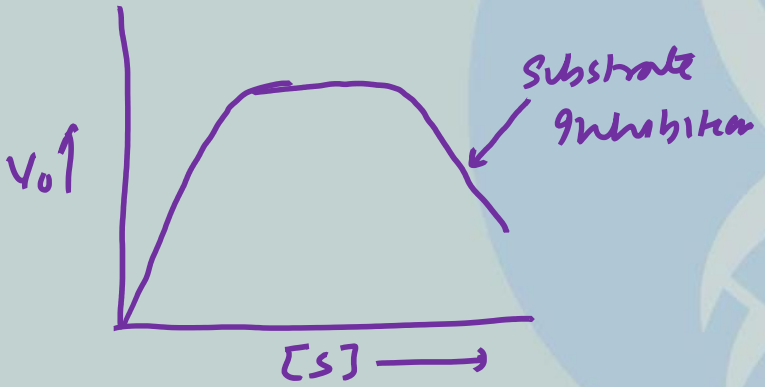


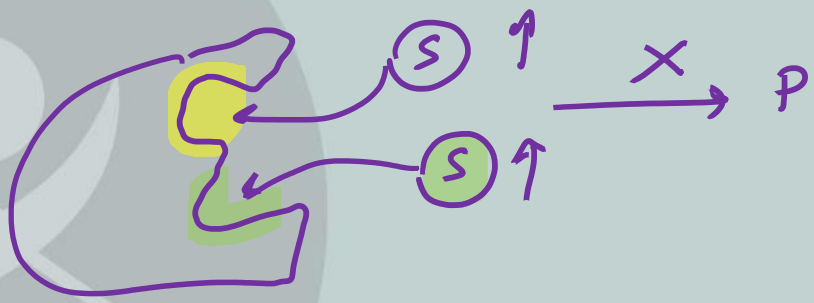
Examples

- Digestive enzymes are secreted as zymogens (pepsinogen, trypsinogen, chymotrypsinogen).
- The coagulation cascade (blood clotting)
- Apoptotic cascade
- Complement protein cascade

4. Substrate Inhibition:

- Enzyme has more than one binding site for the substrate.
- While the first binding site is catalytic, additional binding at a second regulatory site can inhibit the activity of the enzyme





5. Product inhibition:

The product of an enzymatic reaction directly binds to the enzyme's active site, competing with the substrate and preventing further catalysis.

$$E + S \rightleftharpoons ES \rightleftharpoons [EP] \not\rightarrow E + P\uparrow\uparrow$$

- Enzyme is not free

6. Induction and repression of enzyme synthesis → lag time bet^n signal and response

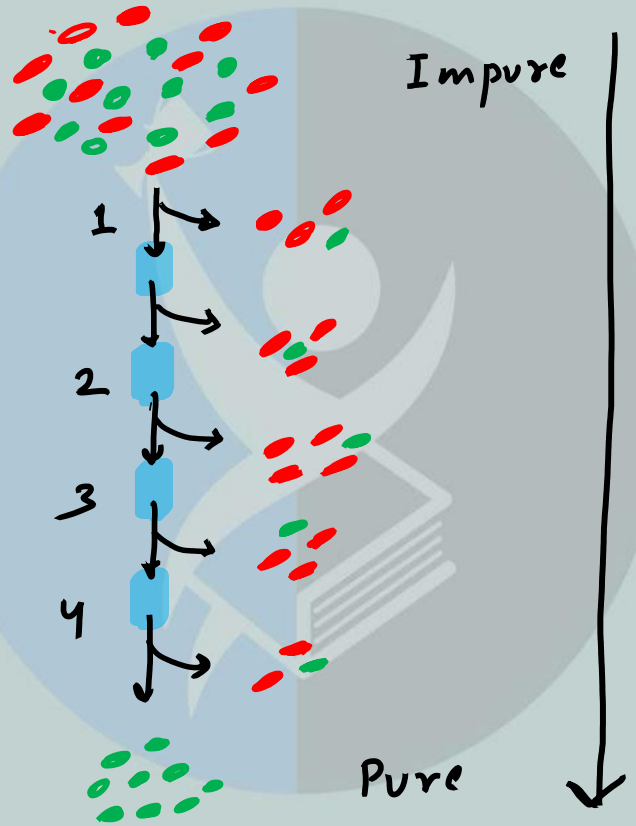
Induction is a process in which the synthesis of a specific enzyme is activated by expression of its gene.

Repression is mechanism by which the synthesis of an enzyme is decreased in response to a specific signal, by inhibiting gene expression.

Basal metabolic Rate (BMR)
- Increase or Decrease

ENZYME PURIFICATION

- Crude enzyme
- Precipitation with ammonium sulphate
- 1
- Ion Exchange Chromatography
- 2
- Gel Filtration Chromatography
- 3
- Affinity Chromatography
- 4
- Packing



Total Activity (Decrease)
% Yield (Decrease)
Specific activity (Increase)
Fold purification (Increase)

Total Activity,(Unit)

- Total activity refers to the overall enzyme activity in a solution at any stage of the purification process.
- Measure of total enzyme in solution

SA

Specific activity (Unit/mg)

- Activity of an enzyme per milligram of total protein.
- Measure of enzyme purity

Yield (%)

- Measure of how much of the initial enzyme activity is retained after each purification step.

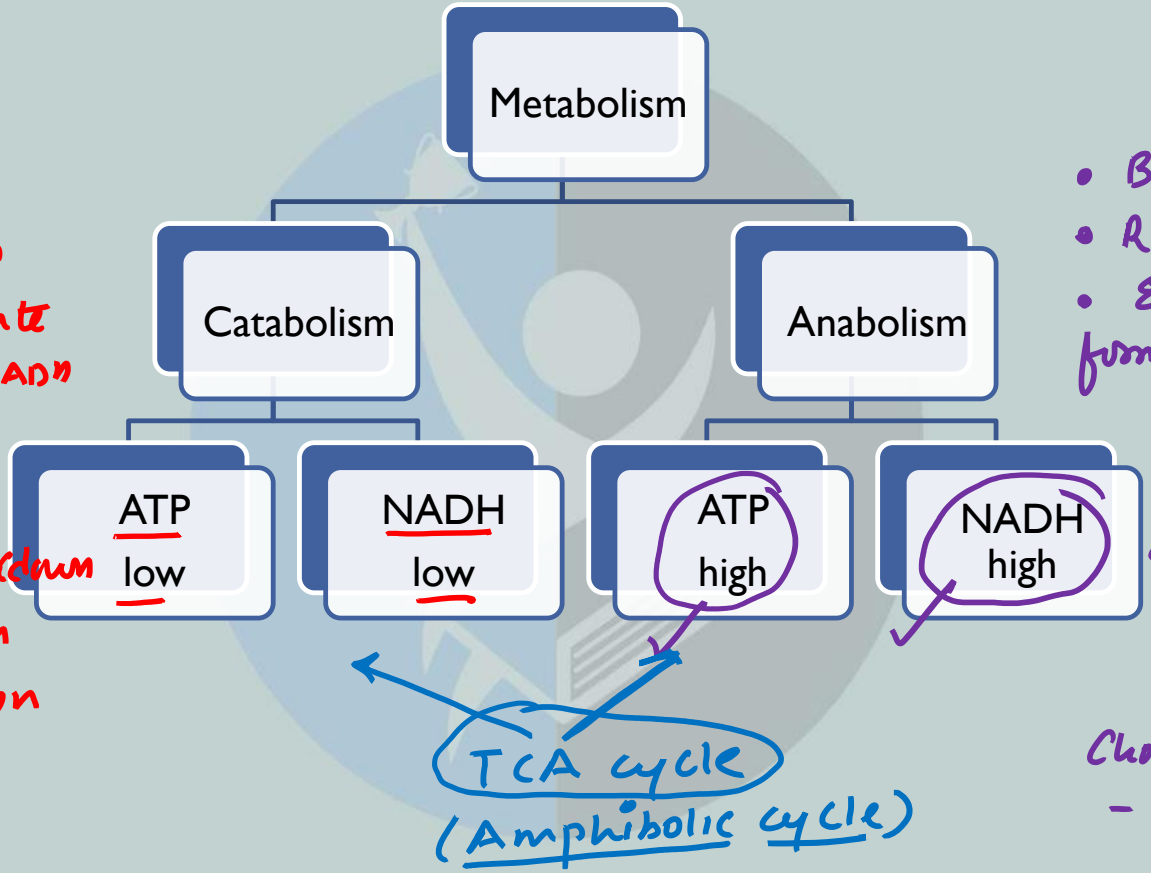
Fold Purification

- Increase in purity from the initial extract to various stages of the purification process
- Calculated by dividing the specific activity after each purification step by the specific activity of the initial extract.

$$= \frac{\text{SA after each step}}{\text{SA of crude enzyme}}$$

	Step	Total Activity (Unit)	Total Protein (mg)	Specific Activity (Unit/mg)	Yield (%)	Fold Purification (X)
1	Protein Extract	10000	1000	$\frac{10000}{1000} = 10$	100%	1
2	Salt Precipitation	9000	600	$\frac{9000}{600} = 15$	90%	$\frac{15}{10} = 1.5$
3	Gel Permeation	~~8000~~ 16000	400	$\frac{8000}{400} = 20$	80%	$\frac{20}{10} = 2$
4	Ion Exchange	7500	250	$\frac{7500}{250} = 30$	75%	$\frac{30}{10} = 3$
5	Affinity Chromatography	7000	100	$\frac{7000}{100} = 70$	70%	$\frac{70}{10} = 7$
		Decrease	Decrease	Increase	decrease	Increase

- If at some step total activity increase it suggest inhibitor was present prior to this step



- Bond Break
- oxidation Rxn
- Energy generate in form ATP, NADH NADPH

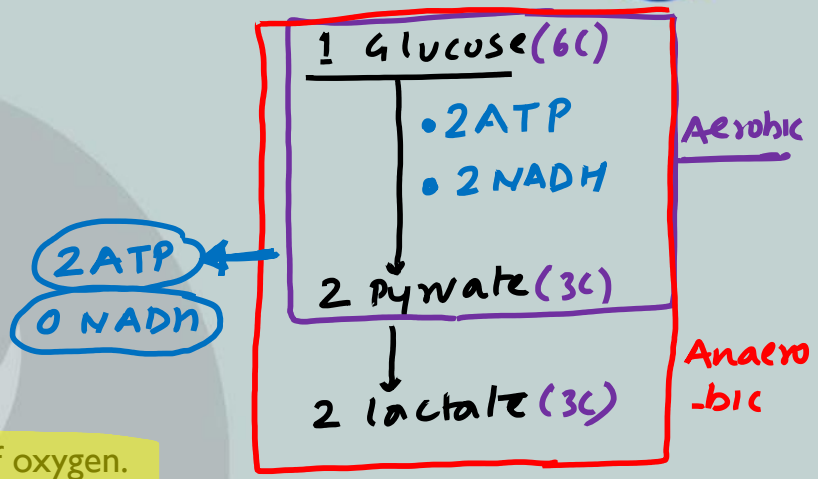
eg Glycolysis
Glycogen breakdown
aa oxidation
FA oxidation

- Bond form
- Reduction rxn
- Energy is used in form of ATP, NADPH or NADH

eg Gluconeogenesis
Glycogen biosynthesis
aa biosynthesis
FA biosynthesis
Cholesterol biosynthesis

GLYCOLYSIS

Embden–Meyerhof–Parnas (EMP) pathway.

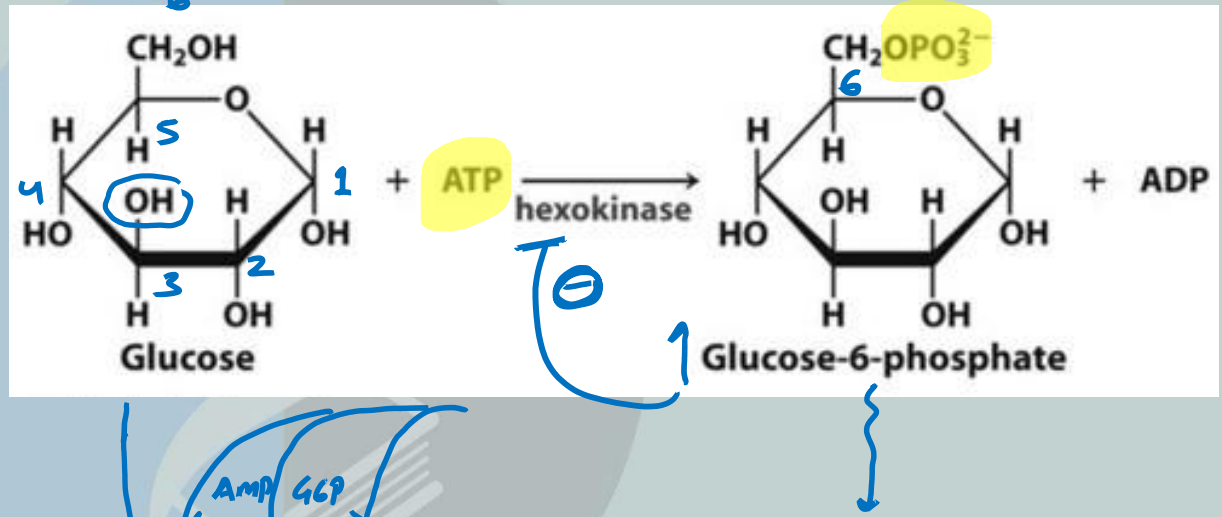


Important features:

1. It is a universal and most conserved pathway
2. Occurs in cytosol all cells of the body- aerobic/anaerobic.
3. Emergency energy-yielding pathway for cells in the absence of oxygen.
4. Major pathway for ATP synthesis in tissues lacking mitochondria, e.g. erythrocytes (RBC), cornea, lens etc.
5. Essential for brain which is dependent on glucose for energy.

Glycolysis

Step 1: Hexokinase: First ATP Utilization



Hexokinase : Most of Tissues

Km for glucose (about 0.1 mM)
Allosteric enzyme
Activator: AMP
Inhibitor: G6P

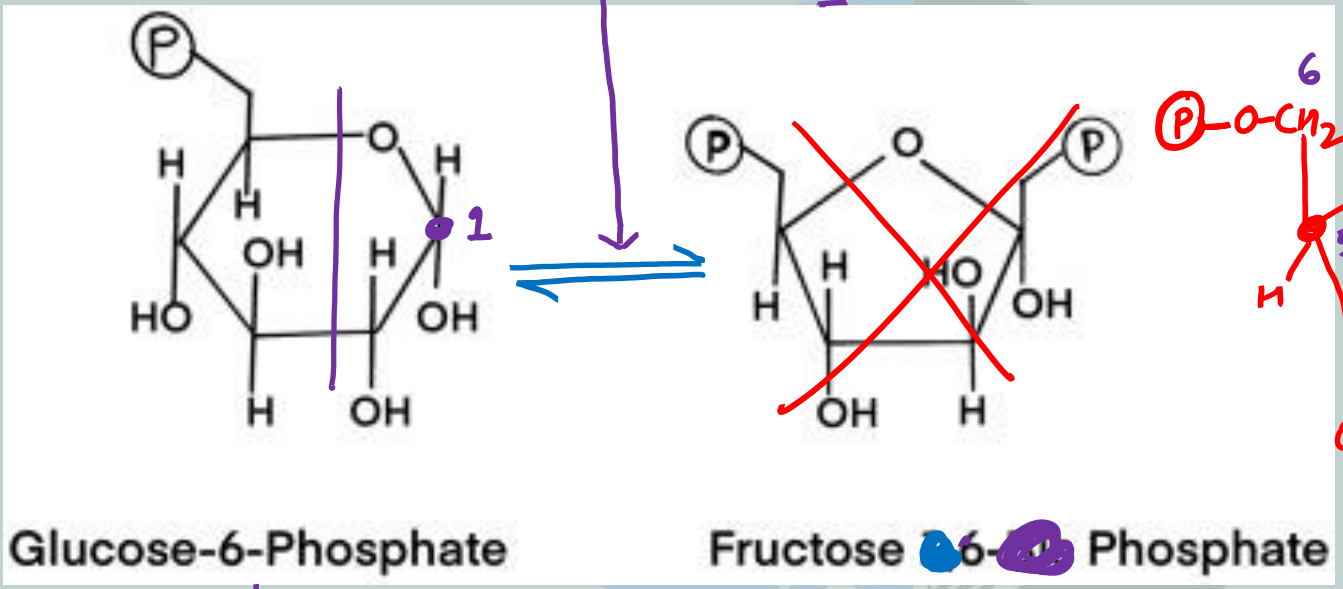
ADP
AMP

Glucokinase: Liver

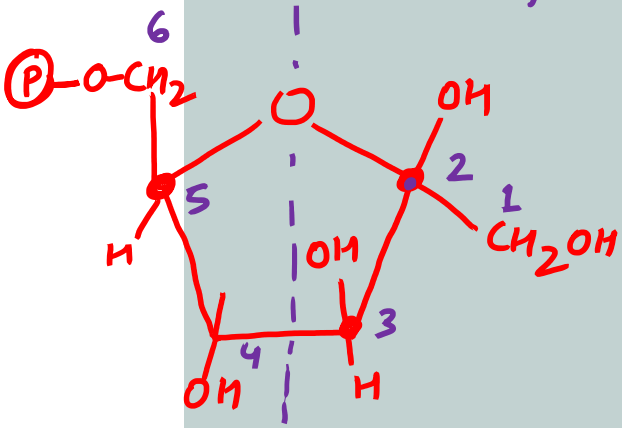
Km for glucose (about 10 mM)
MM enzyme

Low affinity for glucose

Step 2: Phospho-glucose Isomerase



Fructose-6-Phosphate



lysis

4 + 2

lysis

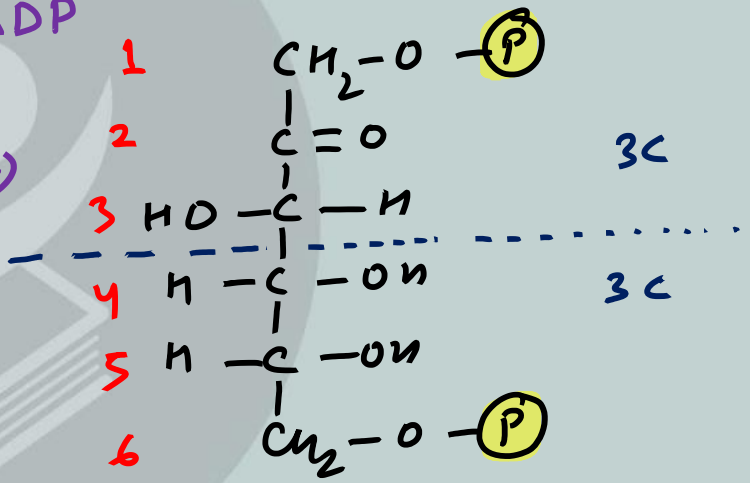
3 + 3

Step 3: Phosphofructokinase: Second ATP Utilization

Fructose-6-P → Fructose 1,6 Bis Phosphate

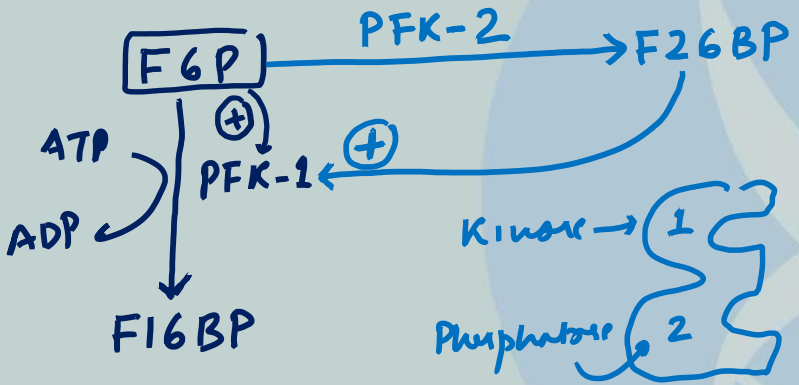
ATP → ADP

- Major Regulatory step
- Pace maker of Glycolysis (slowest)
- Committed step of Glycolysis



Intrinsic Allosteric Regulation of PFK-1: most important

Inhibition: ATP, Citrate, Low pH (H^+)
Activation: AMP, F6P, F2,6 BP, NH_3^+



PFK-2

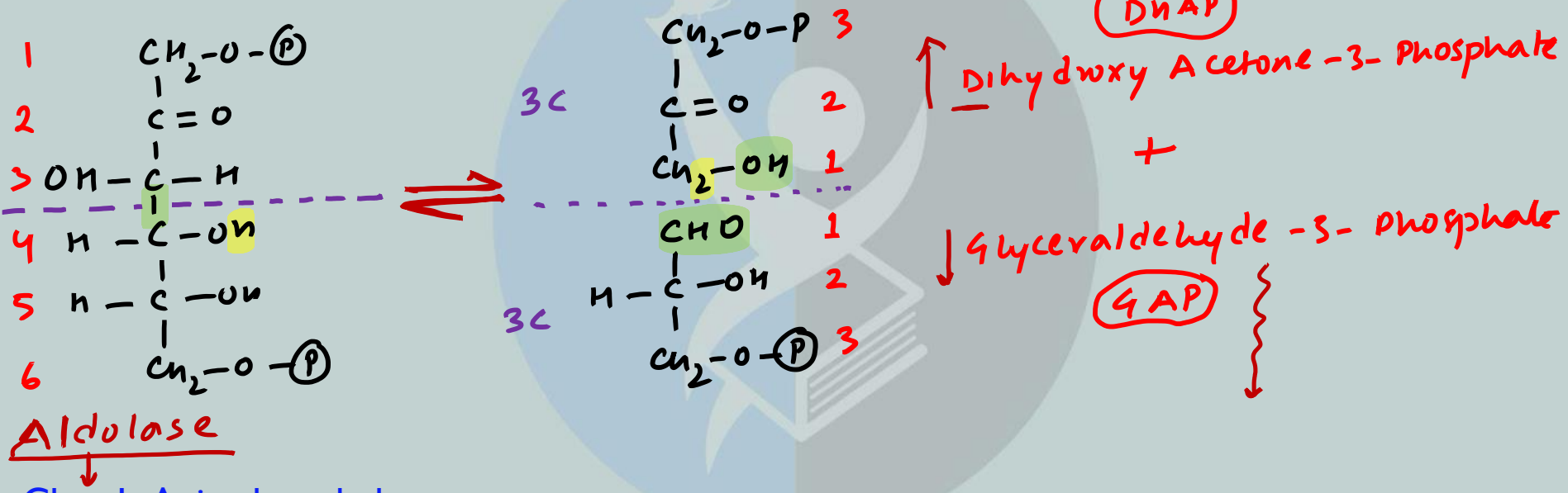
- Single bifunctional enzyme
- Kinase & Phosphatase activity

PFK-2

Inhibitor : Citrate
Activators: F6P, AMP

B. Splitting phase Step

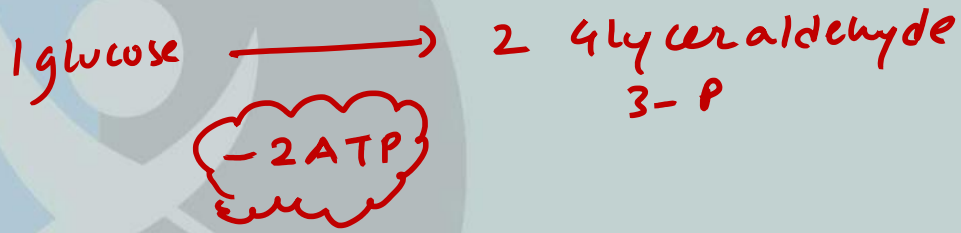
4. Aldolase: Lysis of six carbon compound



Class I : Animals and plants

Class II: Fungi, algae, and some bacteria.

Step 5: Triose Phosphate Isomerase



Inhibitor:

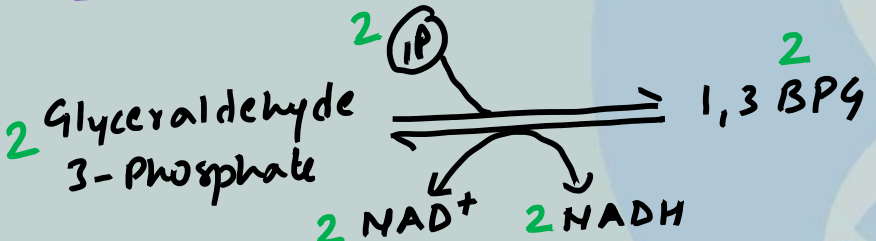
Bromo-hydroxyl-acetone phosphate
Glycidol phosphate.

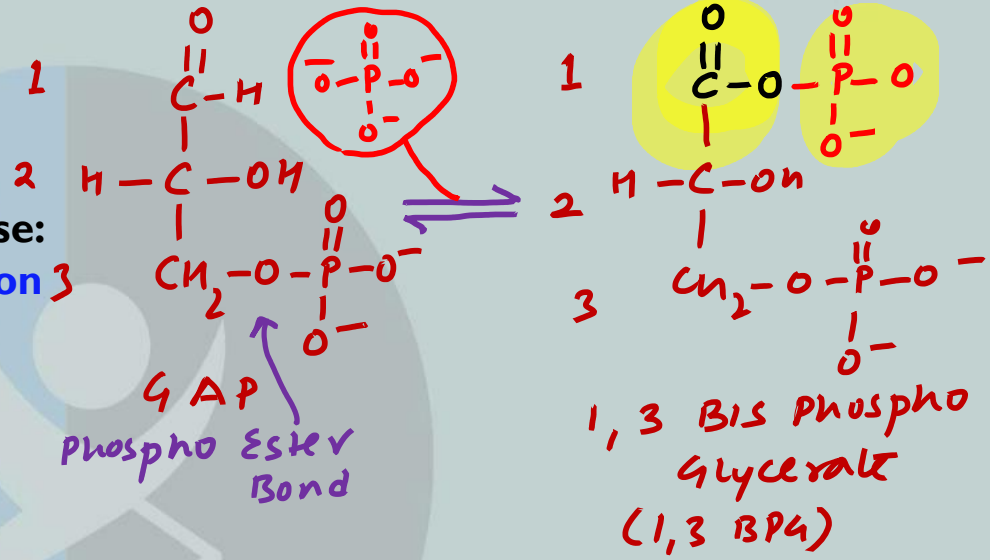
C. Energy generation phase

Step 6: Oxidation

Glyceraldehyde-3-Phosphate Dehydrogenase:
First "High-Energy" Intermediate Formation

1,3 BPG





Inhibitor: Iodoacetate

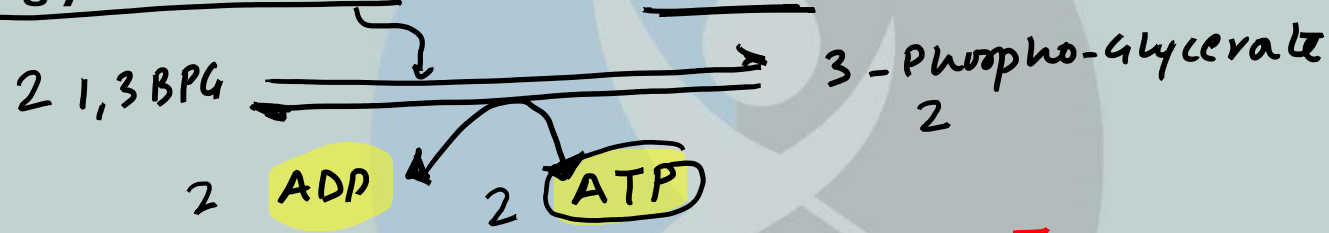
Eg: Irreversible inhibitor

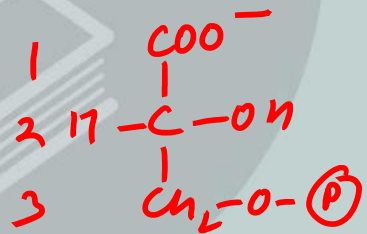
Step 7: Substrate-level phosphorylation

+ 2 ATP

Phosphoglycerate Kinase: First ATP Generation

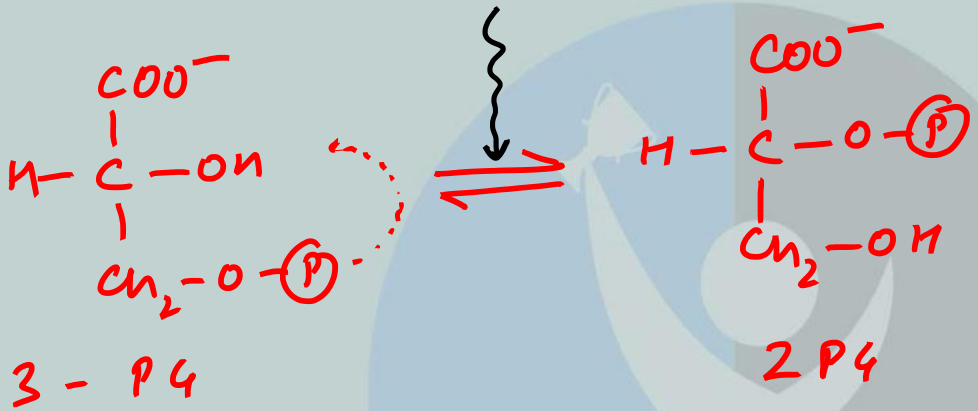
Phosphoglycerate kinase reaction is reversible





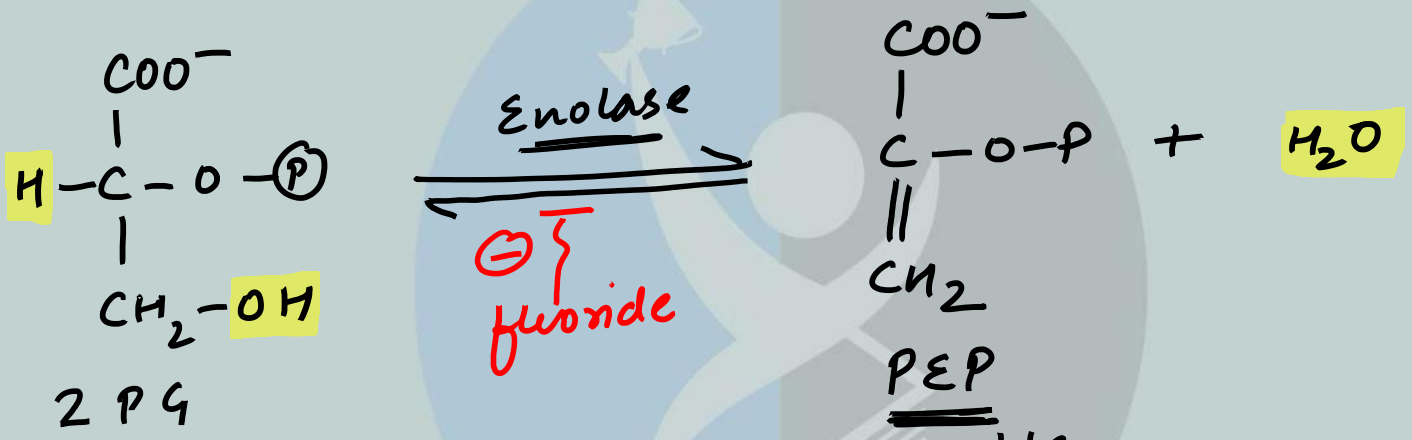
3PG

Step 8: Phosphoglycerate Mutase



Step 9: Enolase:

Second "High-Energy" Intermediate Formation : PEP



PEP

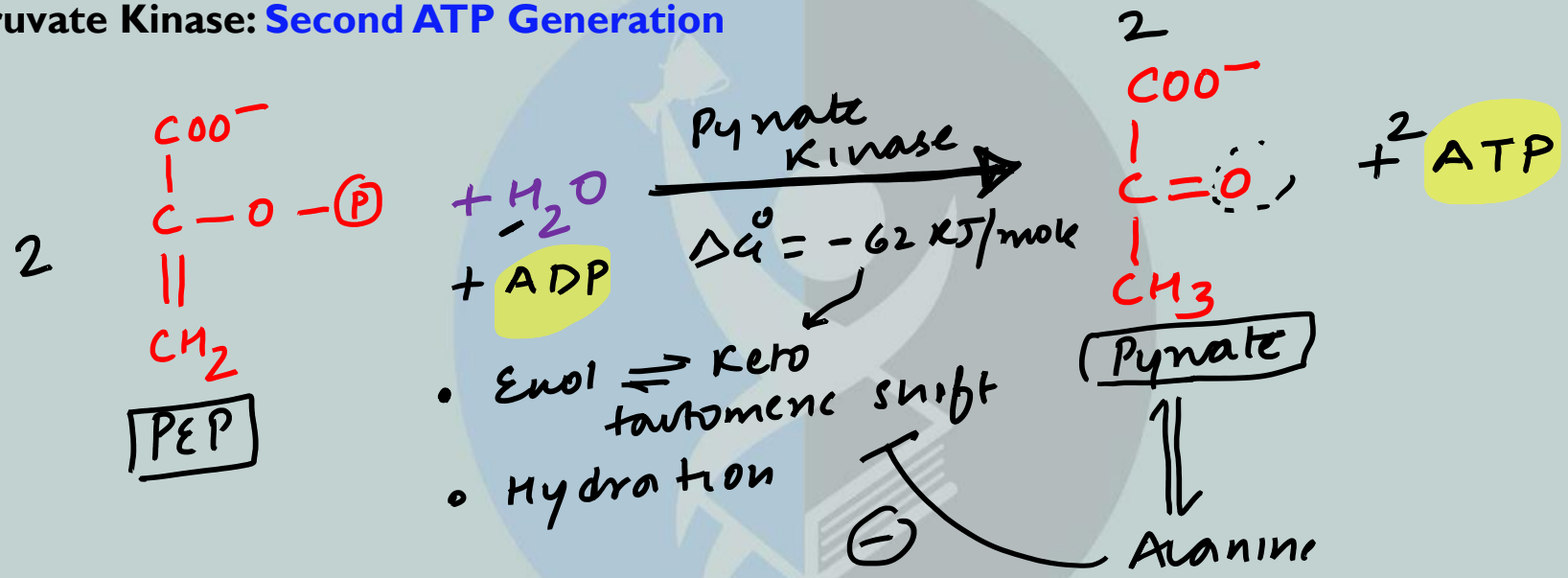
- unstable
- Energy rich bond

Inhibited by fluoride

Step 10: Second Substrate-level phosphorylation

+ 2ATP

Pyruvate Kinase: Second ATP Generation

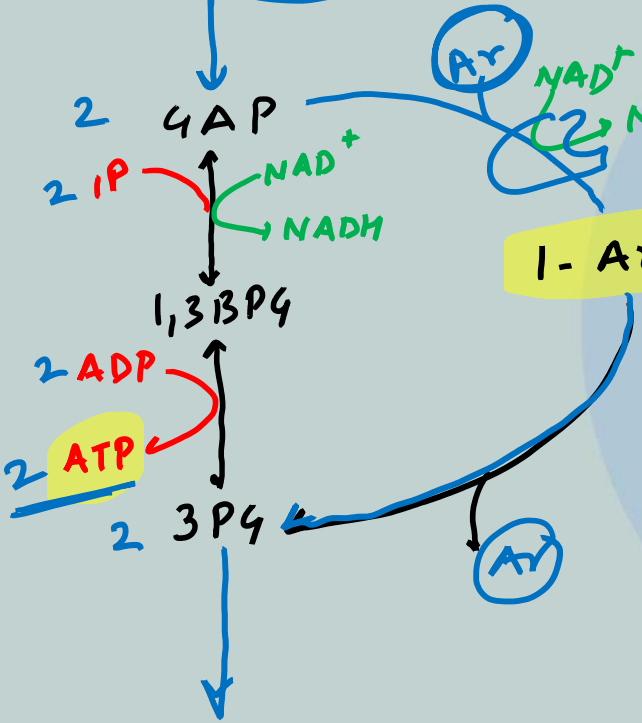


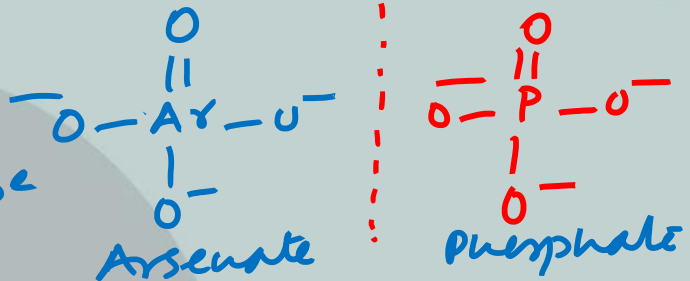
Inhibitor: ATP, Alanine

Activator: AMP, F16BP ← Feed forward activation

Effect of Arsenate

First Substrate level step is bypassed (Net gain 0)





1- Arsino-3- phosphoglycerate

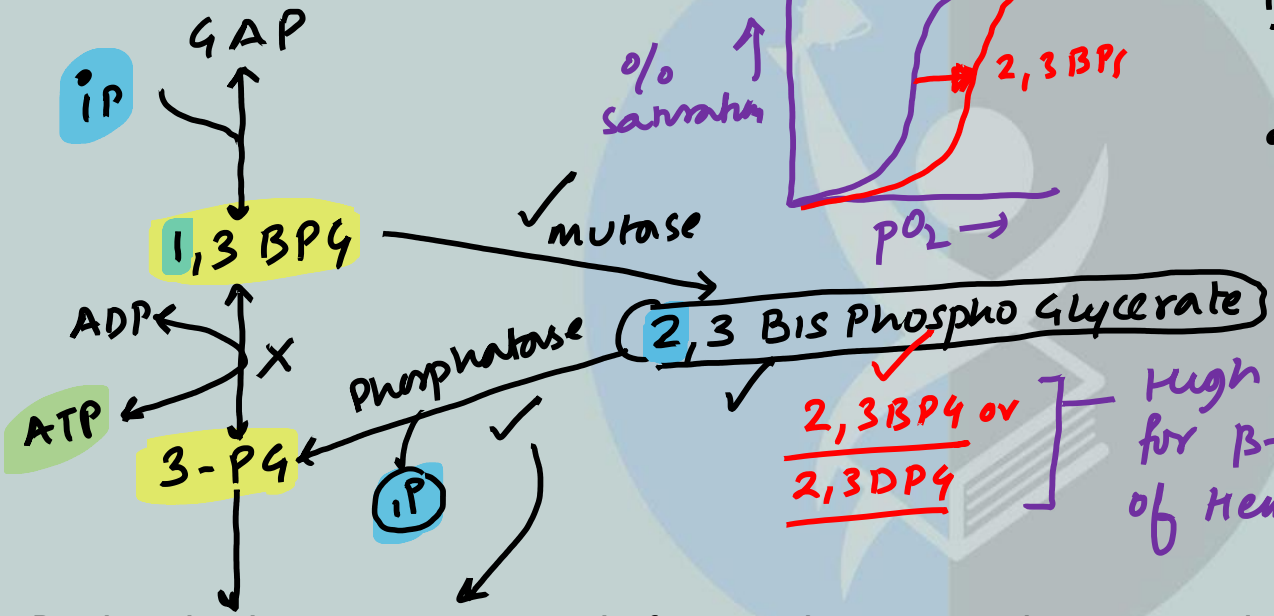
unstable Intermediate

Net gain

- 0 ATP
- 2 NADH

Rapaport-Leubering Cycle of RBC:

First step of ATP synthesis is bypassed and forms 2,3 BPG as intermediate



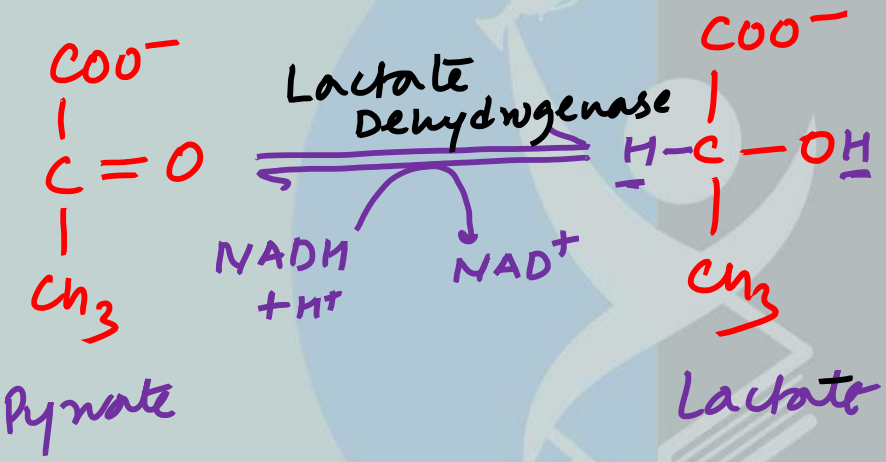
Net gain
- 0 ATP
- 2 NADH

2,3 BPG or 2,3 DPG → High affinity for β-subunit of Hemoglobin → ⊖ cooperativity → oxygen unloading

Bisphosphoglycerate mutase is a bi-functional enzyme with mutase and phosphatase activities

Fates of Pyruvate under anaerobic condition

1. Conversion to lactate (Homolactate fermentation):



- Anaerobic condition in muscles
- RBC (aerobic)
- Warburg effect of cancer cells.

Net gain

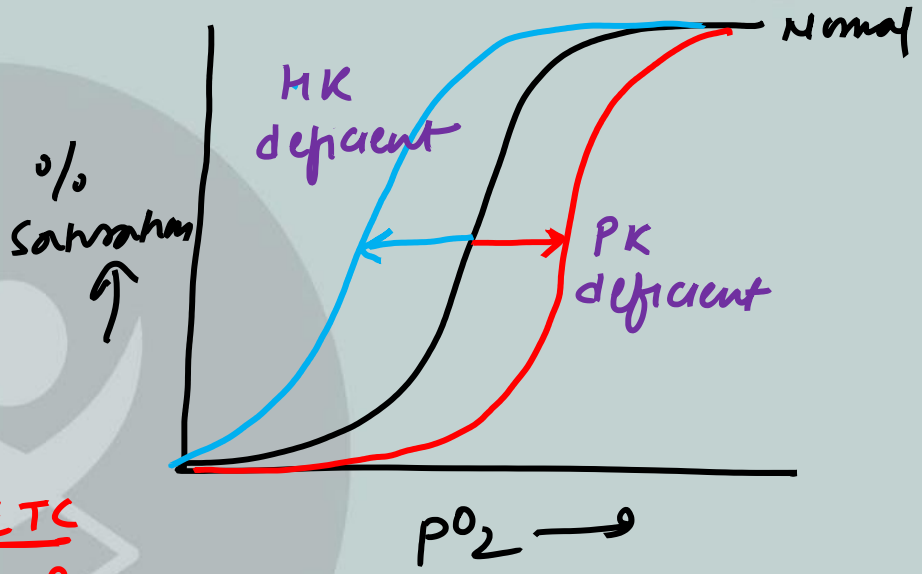
- only 2 ATP

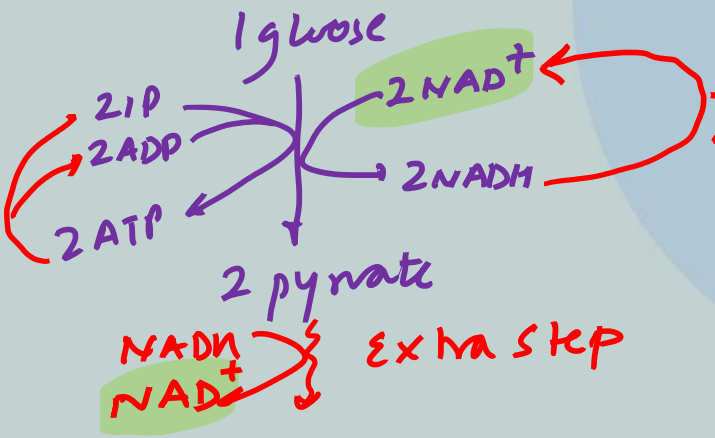
End Product

- 2 lactate

Hexokinase deficient & Pyruvate Kinase deficient:

- NO 2,3 BPG → curve shift to left
- High 2,3 BPG → curve shift to right

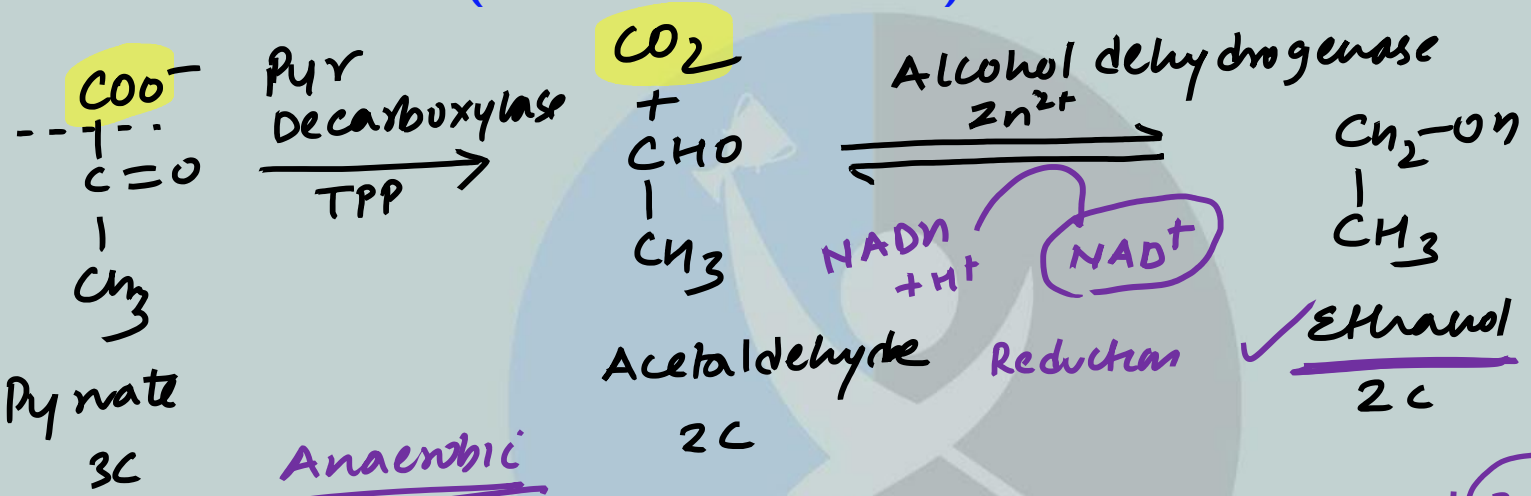




Aerobic : ETC

Anaerobic = ?

2. Conversion to ethanol (Alcoholic fermentation):



Anaerobic

- Yeast
- Fruits

Glucose → 2 ethanol + 2CO2

- 2 ATP

Requirements:	2ADP + 2iP + 2NAD + 1Glucose
Net Gain: (2 Pyruvate)	2ATP + 2 NADH (Aerobic Condition) 2 ATP (Anaerobic Conditions)
3 Regulatory Kinase & Irreversible Steps:	HK (GK), PFK-1, PK
1 Reversible Kinase :	Phosphoglycerate Kinase
1 Oxidation step:	Glyceraldehyde 3 P Dehydrogenase
2 Energy rich molecules:	1, 3 BPG and PEP
2 Substrate level Phosphorylation steps:	1,3BPG→3-PG & PEP→ Pyruvate

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